ONR

ANNUAL PROGRESS REPORT

DATE: May 30, 1991

GRANT NUMBER: N00014-89-J-3027 R&T Code:441d026

PRINCIPAL INVESTIGATOR: Govind S. Nadathur

GRANTEE: The University of California at Santa Barbara

PROJECT TITLE: *Debarymyces hansenii*: A Model System for Marine Molecular Biology

PERIOD OF PERFORMANCE: June 1, 1990 to May 30, 1991

RESEARCH OBJECTIVE: To establish *Debaryomyces hansenii* as a model system for marine molecular biology. Specifically, the objective is to develop plasmid vectors, a transformation system and have a good number of mutants available in this organism.

PROGRESS (YEAR 2)

1. ISOLATION OF AUTONOMOUSLY REPLICATING SEQUENCES (ARS) FROM *DEBARYOMYCES*:

In an attempt to construct plasmids with the capability of autonomous replication we have isolated two ARS from *Debaryomyces* utilizing *S. cerevisiae* strain *SEY 2108 (Ura3⁻)* and its corresponding gene URA3 as a marker. The first, a 400 bp. insert (pAB81) was selected for further analysis. The ARS activity was found to reside in 150 bp. of the clone (pAB83). Sequencing of this region revealed the existence of an AT rich region with the consensus sequence $\overset{A}{T}TTTATRTTT\overset{A}{T}$ which is known to be present in all sequences known to function as ARS in yeast. Attempts are now being made to transform *Ura3⁻ Debaryomyces* with this plasmid. Transformation attempted with techniques such as protoplasting, alkali cations and electroporation has not met with success. This may be an attribute of the strain being used. With the arrival of strain J-26 for Dr. L. Adler, transformation will be attempted utilizing hygromycin resistance as a marker. Dr. Adler has also agreed to generate *Ura3⁻* mutants of his strain for use as a marker.

2. MUTAGENESIS OF *D. HANSENII*:

To generate auxotrophic mutations for transformation, mutagenesis was performed utilizing Ethyl Methyl Sulfonate. *Ura3*$^-$ were selected by the negetive selection technique utilizing 5- fluoro orotic acid. Even though this technique generates a large number of mutants, the stability of the mutants is questionable. Dr. L. Adler, University of Goteborg, Sweden has kindly agreed to send his strain of the organism which apparently has been very successful in mutant generation.

3. ISOLATION OF 17S rRNA AND UBIQUITIN GENES:

To determine the phylogenetic position of *Debaryomyces* relative to *S. cerevisiae* and *Candida albicans* the 17S rRNA and Ubiquitin genes have been isolated with the help of specific oligonucleotide primers and the polymerase chain reaction. The 17s rDNA has now been sequenced and found to have 1798 bp. The phylogenetic comparisons are now being performed.

PUBLICATIONS

1.N. S. Govind and A. Banaszak . Isolation of an Autonomously Replicating Sequence (ARS) from the Halotolerent yeast *Debaryomyces hansenii*. In preparation for *Molecular and Cellular Biology*.

2. N. S. Govind and K. McNally. Isolation and Sequence Analysis of 17s rRNA gene from the marine yeast *Debaryomyces hansenii*. In preparation for *Current Genetics*.

TRAINING ACTIVITIES

Two graduate students, one technician and an undergraduate student have worked varying periods of time on this project to learn the techniques of recombinant DNA technology.

AWARDS / FELLOWSHIPS: None

ANNUAL REPORT QUESTIONNAIRE

Principal Investigator: Dr. Govind S. Nadathur

Institution: University of California, Santa Barbara.

Grant Title: *Debaryomyces hansenii*: A Model System for Marine Molecular Biology.

Period of performance: 7/01/90 to 6/30/91

Number of ONR supported papers: None

Number of ONR supported patents/inventions: None

Number of Presentations:

Invited: 1

Contributed: 1

Number of students: 1
Female: 1
Minority (e.g. Black, Hispanic): 1
Non US citizens: 1

Number of Postdoctoral fellows: None

Awards/Honors to PI and/or to members of the PI's research group (please describe): None

Equipment purchased (# and description of items $1500): None

Your Email Address:None

HIGHLIGHT PAGE

Objectives

1. Construct plasmid for transformation
2. Isolate and Sequence 17s rRNA gene
3. Isolate auxotrophic mutants
4. Isolate Ubiquitin gene

Accomplishments

1. Plasmid with ARS constructed
2. 17s rRNA gene isolated and sequenced
3. Ura3 mutants isolated
4. Ubiquitin gene isolated

Significance

1. Constructed plasmid and mutants to be used for transformation
2. 17s RNA and Ubiquitin genes will determine the position of Debaryomyces among other organisms